EXHIBIT F

ORIGINAL ARTICLE

Dietary N-nitroso compounds and risk of pancreatic cancer: results from a large case–control study

Jiali Zheng[1], Janice Stuff[2], Hongwei Tang[3], Manal M.Hassan[1], Carrie R.Daniel[1] and Donghui Li[3,*]

[1]Department of Epidemiology, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA, [2]USDA Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030, USA and [3]Department of Gastrointestinal Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

*To whom correspondence should be addressed. Tel: +713 834 6690; Fax: +713 834 6153; Email: dli@mdanderson.org
Correspondence may also be addressed to Carrie R. Daniel. Tel: +713 563 5783; Fax: +713 563 1367; Email: cdaniel@mdanderson.org

Abstract

N-nitroso compounds (NOCs) are among the most potent dietary and pancreatic carcinogens. N-nitrosodiethylamine (NDEA) and N-nitrosodimethylamine (NDMA) are the most prevalent NOCs identified in foods. Using a validated and comprehensive N-nitroso database developed to estimate total NOCs and important individual NOCs from food intake, we investigated dietary exposure to NOCs in relation to pancreatic cancer in a large matched case–control study. Self-administered food frequency questionnaires were collected from 957 pathologically confirmed pancreatic ductal adenocarcinoma cases and 938 frequency-matched controls. For each food item, frequency of intake and portion size in grams was multiplied by the estimated NOC concentration from the N-nitroso database. Multiple unconditional logistic regression models were used to estimate the odds ratios (OR) and 95% confidence intervals (CIs) for pancreatic cancer risk by quartiles of NOCs and major food group contributors to NOCs, with the lowest quartile as referent. Following adjustment for confounders, we observed significant positive associations for NDEA ($OR_{Q4\ versus\ Q1}$ = 2.28, 95% CI = 1.71–3.04, P_{trend} < 0.0001) and NDMA from plant sources ($OR_{Q4\ versus\ Q1}$ = 1.93, 95% CI = 1.42–2.61, P_{trend} < 0.0001) with pancreatic cancer. The major food groups related to NDEA and NDMA intakes in this population were fermented cheese, pizza, grains, seafood and beer. No associations of intake of nitrate or total NOCs were observed; nitrite was inversely associated with pancreatic cancer. Although some of our findings probably reflect reverse causation bias due to lower meat intake in cases with latent disease, biologically plausible findings for pancreatic carcinogens, NDEA and NDMA, warrant further prospective investigation.

Introduction

Pancreatic cancer is the fourth leading cause of cancer mortality in the USA among both men and women. Although considered a rare cancer, given its relatively low incidence, it has the highest case fatality rate among major cancers (1). Identifying modifiable risk factors for this malignancy is urgent and of significant public health importance. Although some well-recognized risk factors including age, body fatness, adult attained height, cigarette smoking, diabetes and family history have been unraveled, the role of diet in etiology of pancreatic cancer is not clear (2).

Animal studies have provided strong evidence for the carcinogenicity of N-nitroso compounds (NOCs) on many different organs, including the pancreas (3). NOCs are classified among the most potent dietary carcinogenic agents and consist of two main groups: N-nitrosamines and N-nitrosamides. Among the several hundred NOCs that haven been examined, N-nitrosodiethylamine (NDEA) and N-nitrosodimethylamine (NDMA) are the most prevalent compounds in foods (4). Because of limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals, NDEA

Case 1:19-md-02875-RMB-SAK   Document 1716-8   Filed 11/01/21   Page 3 of 10
PageID: 42335
J. Zheng *et al.* | 255

Abbreviations

BMI	body mass index
CI	confidence interval
FFQ	food frequency questionnaire
NDBA	N-nitrosodibutylamine
NDEA	N-nitrosodiethylamine
NDMA	N-nitrosodimethylamine
NPYR	N-nitrosopyrrolidine
NOC	N-nitroso compound; OR, odds ratio

and NDMA were classified as class 2A carcinogens, i.e. probable human carcinogens by International Agency for Research on Cancer (5).

Human exposure to NOCs includes both preformed and endogenous NOCs. Preformed NOCs occur through use of tobacco products, food consumption, occupational exposures and other miscellaneous minor sources (6). It is estimated that 45–75% of total NOC exposures are synthesized endogenously from nitrite's reaction with the degradation products of amino acids in the stomach (7). Food cured with nitrate and/or nitrite (e.g. processed meat), pickled, stored under humid conditions, smoked in air saturated with nitrogen or dried at high temperatures (i.e. beer, non-fat dry milk, cooked bacon or dried meats) are those with the highest concentrations of NOCs (8).

Although the carcinogenicity of processed meat has garnered significant attention recently (9), findings from epidemiological studies investigating the associations of dietary NOC exposure or consumption of NOC-rich foods, e.g. processed meat, with pancreatic cancer are inconsistent (2,10). For the assessment of NOC exposure, nearly all studies focused on total NOCs, nitrate and/or nitrite only. Among three case–control studies, two reported inverse associations for nitrate (11,12), one reported a positive association for nitrite (10) and one reported null associations for nitrate and nitrite (13). Among four prospective cohort studies, no statistically significant associations were observed for intake of nitrate or nitrite and pancreatic cancer risk (14–17). However, two of the cohorts showed some evidence that intake of nitrite from processed meat may increase risk of pancreatic cancer (14,15).

To further examine the role of specific NOCs in pancreatic cancer risk, we used a validated and relatively comprehensive NOC database encompassing 21 different individual compounds as well as nitrate and nitrite (18) with consideration of animal and plant sources, separately. Previous evidence suggests two dietary nitrosation inhibitors, vitamin C and E, inhibit endogenous NOC formation (19), whereas heme iron in red meat stimulates endogenous NOC formation (20). Apart from diet, tobacco products represent another major environmental exposure contributing to exogenous NOCs (4). Thus, in our study we additionally assessed whether dietary intake of vitamin C, E or red meat, and cigarette smoking modified associations of dietary NOCs with pancreatic cancer risk.

Materials and methods

Study design and study population

The study design and study population have been described in detail previously (21). Briefly, this is a hospital-based case–control study of pathologically confirmed pancreatic ductal adenocarcinomas recruited from the Gastrointestinal Cancer Center at the University of Texas MD Anderson Cancer Center (MD Anderson). Controls were cancer-free individuals recruited from friends and genetically unrelated family members (usually spouses or in-laws) of patients diagnosed with cancers other than pancreatic cancer, lung cancer or head and neck cancer

(i.e. smoking-related cancers) (21). Cases and controls were recruited simultaneously and consecutively during January 2002 and June 2009 with no restriction in terms of age, race and sex (22). The response rates for case and control recruitment were both ~78% with no significant differences in demographic factors between individuals who agreed or declined to participate in the study (22). Previous analysis results indicated that cases and controls were from the same catchments, which supports the idea that controls were representative of the hospital population from which the pancreatic cancer cases were drawn (21). This study was approved by the institutional review board of MD Anderson. Informed consent was obtained from each participant in this study. We removed participants with improbable dietary data via evaluation of sex-specific outliers (i.e. two interquartile above 75th percentile or below 25th percentile) of BOX-COX transformed total energy intake (*n* = 5) (23); and who left more than half of the food frequency questionnaire (FFQ) food items blank (*n* = 31), which resulted in a total of 1110 cases and 1010 controls. We then performed frequency matching on age group, race and sex; and of these, the final matched dataset for analysis included 957 cases and 938 controls.

Data collection

Each study participant was interviewed by well-trained interviewers who followed a written protocol to collect information on demographic features, risk factors such as smoking history, alcohol use, family history of cancer and medical history using a structured and validated questionnaire (21). No proxy interviews were conducted. Body mass index (BMI) was calculated as weight (kg)/height (m²) and categorized based on the World Health Organization criteria. Participants self-reported their body weight values at different stages of life from 14–19 years of age to older than 70 years of age across 10-year age intervals with the final reported weight within the year prior to their recruitment. BMI was calculated using weight at each age period and the self-reported adult height. Because of the expected high prevalence of prediagnostic weight loss in patients with pancreatic cancer, we chose BMI in the 30s as the primary covariate of interest given its previously observed associations with pancreatic cancer risk in this study (24). Alcohol consumption was estimated and categorized using number of alcoholic drinks per day based on the alcohol content level in a standard drink in the USA (0, 0–3 and >3 drinks/day) (25).

Dietary assessment

Over the course of study recruitment, two different versions of Harvard semi-quantitative FFQ were used to assess self-reported diet in the previous year. A total of 1729 individuals (776 cases and 953 controls) completed the original version of the FFQ whereas 391 individuals (334 cases and 57 controls) completed an updated version. The original and updated FFQs covered 84 and 131 food items, respectively. Each questionnaire asked additional questions about the types of fat used for cooking, the form and type of margarine used, the amounts of sugar added to food, the usual brand and type of cold breakfast cereal, and dietary supplements (26). The updated FFQ additionally included changes to the consumer food market, such as adding soy milk and extreme lean hamburger, and incorporated open-ended questions to identify other important foods consumed at least once per week. On both questionnaires, portion size was specified using natural units whenever possible or otherwise based on analyses of diet records (e.g. 1/2 cup of string beans or 4–6 oz of meat as a main dish) (27). The frequency of intake of the specified portion size of each food was asked with nine multiple-choice responses, ranging from never/less than once per month to six or more times per day. For both original and updated versions of the Harvard FFQ, validity was tested against a 4-week and two 1-week diet records among a small subsample in the Nurses' Health Study and Health Professionals Follow-Up Study, respectively, which supported modest correlation coefficients between the energy-adjusted nutrients intake measured by diet records and the FFQ (*r* ranged from 0.36 to 0.75 for the original version and ranged from 0.28 to 0.86 for the updated version) (26,28).

Calculation of NOCs, nitrate and nitrite values

We used an N-nitroso database consisting of 21 different NOCs as well as nitrate and nitrite (i.e. a total of 23 items) for 500 foods from 39 food groups (18) to calculate the NOCs and nitrate and nitrite values in our study.

This database was developed originally in conjunction with an existing modified Block FFQ established based on the National Cancer Institute's Health Habits and History Questionnaire, to assess dietary NOCs in cancer studies (18,29). The details of the construction of this database are described elsewhere (18). Briefly, the *N*-nitroso database was constructed through a comprehensive internet search of assays that reported for NOCs and research publications on food composition and food assays as well as reports from government agencies. In this database, each food group formed from aggregating individual foods with similar usage and nutrient composition received the same NOC value for the purpose of easy application in the linkage with foods from different FFQs. Food groups in this database consisted of those with high concentrations of NOCs such as sausage, bacon and ham, and foods with lower concentrations such as vegetables and grains (18). The validity of this database has been assessed by comparing NOCs consumption derived from the modified Block FFQ with 7-day food records in a cross-sectional study of 98 healthy controls, and findings showed modest agreement from the two methods (30).

To estimate the dietary intake of NOCs and nitrate and nitrite, we first translated portion size to weight for each food item and multiplied by the frequency of intake to derive the amount of food consumed per day (31). FFQ-specific calculations were conducted to account for different portion-to-gram translation and non-overlapping food items between the two versions. We then calculated the amount of individual NOC, nitrate and nitrite intake from each food item by multiplying the consumption amount in grams of the food item and the concentration of NOC, nitrate and nitrite in the corresponding food. Finally, the daily intake of each NOC was derived by summing the *N*-nitroso values contributed by all foods. The daily total NOC intake was the sum of all 21 individual NOCs plus nitrate and nitrite.

Statistical analysis

The descriptive statistics for demographic and pancreatic risk factors among cases and controls were calculated with frequencies and percentages. Difference between cases and controls were evaluated using chi-square test. To assess the associations of pancreatic cancer with consumption of major food groups that contributed to NOCs (red and processed meat, total vegetables and green leafy vegetables) and dietary intake of total and individual NOCs, nitrate and nitrite, each variable of interest was divided into quartiles based on the log-transformed energy-adjusted cutoff points of the control distribution. The FFQ-specific quartiles were used in the unconditional logistic regression to estimate the multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for pancreatic cancer risk with subjects in the lowest quartile as reference (32). In the multivariable-adjusted model, we adjusted for total energy intake as continuous variable; and age group, sex, race, education level, BMI status, alcohol level, history of diabetes, smoking status and family history of pancreatic cancer as categorical variables as defined in Table 1. These variables were selected *a priori* based on previous supporting literature (33–35). For the food group analysis, we also mutually adjusted for other meat groups (e.g. red meat and white meat, processed and non-processed meat). Fruit and vegetable group and meat group were adjusted simultaneously for each other as well to account for the possible residual confounders (35). NOCs from plant foods and animal foods were investigated separately with mutual adjustment for intake of red and processed meat in the NOCs from plant foods analysis and mutual adjustment for total fruits and vegetables in the NOC from animal foods analysis. Linear trend was assessed across the median value of each quartile (modeled as a continuous variable) in the multivariable-adjusted model.

We investigated the role of dietary vitamin C and E intake (low and high level), red meat intake (low and high), cigarette smoking (never, former and current smokers), alcohol consumption (0, 0–3, >3 drinks/day) and pancreatic cancer family history (yes, no) in modifying the association between NOC and pancreatic cancer risk. Tests for interaction were performed by adding the cross-product of the two-level NOCs and each categorical effect modifier in the multivariable-adjusted model with a *P*-value ≤0.1 as an indicator of potentially significant interaction (36).

Several sensitivity analyses were also conducted. First, owing to a large missing percentage of BMI at age 30s in our dataset, we reran all analyses excluding individuals with missing BMI values. Second, we conducted an analysis restricted to individuals without diabetes, as diabetes diagnosis may result in diet modification (37). We also restricted our analyses to participants who completed the original version of the FFQ to examine if inclusion of the updated FFQ in a smaller subset of participants had an effect on our overall findings.

We additionally assessed the top five major food group contributors to NOC intake by calculating Spearman correlation coefficients between each energy-adjusted NOC and food group in the *N*-nitroso database in cases and controls separately.

All statistical analyses were conducted using SAS® (version 9.4; SAS, Cary, NC). All tests were two-sided with *P* values <0.05 considered to be statistically significant if not otherwise noted.

Results

Table 1 shows characteristics of the study participants in the matched study design. As compared with controls, cases tended to have less than a college education and were more likely to be current or former smokers who quit <10 years. Cases were also more likely to be diabetic, overweight or obese in their 30s; have family history of pancreatic cancer and consume >3 alcoholic drinks per day.

Among the 21 individual NOCs analyzed in this study, 11 NOCs came from very limited number of foods (18) consumed by our participants; and thus were not carried forward in the analysis. For the 10 remaining individual NOCs including *N*-nitrosoamino acids (NAA), *N*-nitrosodibutylamine (NDBA), NDEA, NDMA, *N*-nitrosomorpholine (NMOR), *N*-nitrosopiperidine (NPIP), nitrosoproline (NPRO), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosothiazolidine-4 carboxylic-acid (NTCA) and *N*-nitrosothiazolidine (NTHZ), we reported associations with pancreatic cancer for NDEA, NDMA, NDBA and NPYR in Table 2. Data on the remaining six NOCs are presented in Supplementary Tables 1 and 2, available at *Carcinogenesis* Online. NMOR and NPIP were very limited in intake and range and NAA, NPRO, NTCA and NTHZ were not classified in their carcinogenicity to humans (5).

In the multivariable-adjusted model, a higher risk of pancreatic cancer was observed for the highest dietary intake of NDEA from plant sources ($OR_{Q4 \text{ versus } Q1}$ = 1.93, 95% CI = 1.44–2.60, P_{trend} < 0.0001) and from animal sources ($OR_{Q4 \text{ versus } Q1}$ = 1.35, 95% CI = 1.03–1.78, P_{trend} = 0.004), as well as total NDEA from both animal and plant sources ($OR_{Q4 \text{ versus } Q1}$ = 2.28, 95% CI = 1.71–3.04, P_{trend} < 0.0001). Notably, a positive association was detected for NDMA from plant source ($OR_{Q4 \text{ versus } Q1}$ = 1.93, 95% CI = 1.42–2.61, P_{trend} < 0.0001) but not for NDMA from animal sources. No association was found for nitrate from either plant or animal sources. Nitrite and NDBA consumption in our study were all from animal sources and were both inversely related to pancreatic cancer (nitrite: $OR_{Q4 \text{ versus } Q1}$ = 0.68, 95% CI = 0.51–0.91, P_{trend} = 0.01; NDBA: $OR_{Q4 \text{ versus } Q1}$ = 0.64, 95% CI = 0.48–0.82, P_{trend} = 0.003). Similarly, greater intake of NPYR from animal sources was inversely associated with pancreatic cancer ($OR_{Q4 \text{ versus } Q1}$ = 0.77, 95% CI = 0.58–1.02, P_{trend} = 0.04). The sum of all 23 different NOC-related compounds in the *N*-nitroso database, either from animal sources or from plant sources, was not associated with pancreatic cancer. No change in significance level was observed and ORs did not change materially in any of the food group and NOCs sensitivity analyses we performed (data not shown).

In the stratified analyses, we did not observe significant effect modification by dietary vitamin C, E or red meat intake, or by alcohol intake level (data not shown). A stronger positive association was observed for NPIP and pancreatic cancer among current smokers ($P_{interaction}$ = 0.05) compared with never and former smokers whereas a positive association was only seen for NAA and pancreatic cancer among never smokers, but not

Case 1:19-md-02875-RMB-SAK   Document 1716-8   Filed 11/01/21   Page 5 of 10
PageID: 42337
J.Zheng *et al.* | **257**

Table 1. Demographic characteristics and risk factors for pancreatic cancer among 1895 matched cases and controls on age groups, race and sex

	Cases (n = 957)	Controls (n = 938)	P value[a]
Age groups (years)[b,c]			0.28
<50	108 (11.3)	125 (13.3)	
50–59	253 (26.4)	268 (28.6)	
60–69	386 (40.3)	348 (37.1)	
≥70	210 (21.9)	197 (21.0)	
Race[b]			0.18
White	856 (89.5)	855 (91.2)	
Hispanic	51 (5.3)	51 (5.4)	
African American	41 (4.3)	29 (3.1)	
Other	9 (0.9)	3 (0.3)	
Sex[b]			0.38
Males	566 (59.1)	536 (57.1)	
Education level			0.001
Less than or equal to high school	243 (25.4)	198 (21.1)	
Some college/technical or vocational school	253 (26.4)	294 (31.3)	
College graduate	233 (24.4)	273 (29.1)	
Postgraduate	224 (23.4)	171 (18.2)	
Missing	4 (0.4)	2 (0.2)	
History of diabetes[c]			<0.0001
No diabetes history	716 (74.8)	838 (89.3)	
Yes, shorter duration (<6 years)	166 (17.4)	46 (4.9)	
Yes, longer duration (≥6 years)	71 (7.4)	49 (5.2)	
Yes, unknown duration	1 (0.1)	5 (0.5)	
Unknown status	3 (0.3)	0 (0)	
Smoking status[c]			<0.0001
Non-smokers	427 (44.6)	494 (52.7)	
Former smokers, quit <10 years	60 (6.3)	48 (5.1)	
Former smokers, quit ≥10 years	201 (21.0)	275 (29.3)	
Former smokers, unknown quit years	29 (3.0)	6 (0.6)	
Current smokers, pack-years <21	94 (9.8)	40 (4.3)	
Current smokers, pack-years ≥21	143 (14.9)	73 (7.8)	
Missing smoking status	3 (0.3)	2 (0.2)	
BMI at age 30s (kg/m^2)[c]			<0.0001
Underweight	13 (1.4)	23 (2.5)	
Normal	393 (41.1)	391 (41.7)	
Overweight	246 (25.7)	201 (21.4)	
Obese	81 (8.5)	39 (4.2)	
Missing	224 (23.4)	284 (30.3)	
Family history of pancreatic cancer			0.0001
Yes	57 (6.0)	20 (2.1)	
No	892 (93.2)	912 (97.2)	
Missing	8 (0.8)	6 (0.7)	
Alcohol drinks per day			<0.0001
0	406 (42.4)	426 (45.4)	
0–3	396 (41.4)	433 (46.2)	
>3	152 (15.9)	79 (8.4)	
Missing	3 (0.3)	0 (0.0)	

[a]P value was calculated with chi-square test.
[b]Matching factors in this study.
[c]Percentage may not add up to 100% for cases and controls because of rounding.

among current or former smokers ($P_{interaction}$ = 0.05). It is possible these significant interactions are chance finding due to small numbers of cases within some strata.

To better understand some of the unexpected associations we observed in our analyses between NOC intake and pancreatic cancer, we investigated the associations of NOC food group sources with pancreatic cancer. As shown in Table 3, after adjusting for confounders, high red meat intake was inversely associated with pancreatic cancer ($OR_{Q4 \text{ versus } Q1}$ = 0.65, 95% CI = 0.48–0.86, P_{trend} = 0.005) with similar associations observed for total red and processed meat ($OR_{Q4 \text{ versus } Q1}$ = 0.62, 95% CI = 0.47–0.82, P_{trend} = 0.003). However, no association was observed for total processed meat or processed red meat. An inverse association was also observed for total vegetable intake ($OR_{Q4 \text{ versus } Q1}$ = 0.75, 95% CI = 0.56–1.00, P_{trend} = 0.04) and leafy vegetables ($OR_{Q4 \text{ versus } Q1}$ = 0.74, 95% CI = 0.55–0.99, P_{trend} = 0.04). Correlation analysis showed that the primary contributors to nitrite, NDBA and NPYR intakes included red and processed meats such as beef, cured lunch, hot dog or bacon. For NDEA and NDMA, the most significant food sources included fermented cheese, pizza, seafood, grains and beer (Table 4).

Discussion

In this large matched case–control study of pancreatic cancer, we used a comprehensive NOC database to investigate the associations of individual and combined NOCs with pancreatic cancer. A significant positive association was found for NDEA and NDMA, the two most prevalent dietary NOC carcinogens. No significant association was observed for nitrate and total NOCs. An inverse association was observed for nitrite, NDBA and NPYR. These findings support our hypothesis that exposure to specific dietary NOCs modifies the risk of pancreatic cancer and warrant deeper investigation of NOC compounds in prospective and mechanistic studies.

The most compelling and biologically plausible finding of this study is the significant positive associations of NDEA and NDMA with risk of pancreatic cancer. NDEA and NDMA are the most abundant NOCs identified in foods (4) and are potent pancreatic carcinogens in animal models. NDEA could directly bind to DNA to form DNA alkylation products, and human pancreatic cells can activate NDMA to their ultimate carcinogenic forms (6). Chronic exposure to NOCs could potentially overwhelm DNA repair capabilities and the unrepaired DNA alkylation damage would lead to gene mutation and cancer development (6). The major food sources of NDEA and NDMA are protein-containing foods dried at high temperatures, e.g. beer ingredients, non-fat dry milk, cooked bacon, or dried meats, or foods preserved with nitrite, e.g. cured, smoked or pickled meats and fish (10). The major food groups that contributed to the NDEA and NDMA intakes in the current study were fermented cheese, pizza, seafood, beer and grains. Although cases consumed less red and processed meat than controls in this study, which could drive the risk estimates toward to null, a significantly higher intake of fermented cheese was found among cases compared with controls (P = 0.003). NDEA and NDMA could also be formed endogenously through acid-catalyzed or bacterially catalyzed N-nitrosation in the stomach. Both NDEA and NDMA have been detected in the gastric juice of subjects after overnight fasting (10). Interestingly, a previous study has found that certain bacterial strains contained in fermented foods play a

Table 2. Multivariable-adjusted ORs and 95% CIs of pancreatic cancer risk according to quartiles of consumption of N-nitroso compounds (µg per 1000 kcal/day)

N-nitroso compounds from dietary sources	Quartiles of intake[a]				P_{trend}[b]
	Q1	Q2	Q3	Q4	
Total N-nitroso compounds[c]					
Mean (range)	22.53 (4.39–30.07)	35.23 (28.72–41.13)	46.45 (38.42–54.30)	74.19 (48.39–287.8)	
Case/control	255/235	234/234	197/235	271/234	
Age and energy-adjusted OR (95% CI)	1.00	0.94 (0.73–1.21)	0.79 (0.61–1.02)	1.09 (0.85–1.41)	0.47
Multivariable-adjusted OR (95% CI)[d]	1.00	0.94 (0.71–1.23)	0.75 (0.56–0.99)	1.08 (0.82–1.43)	0.58
Plant sources					
Mean (range)	18.98 (0.56–26.60)	31.44 (24.69–37.26)	42.59 (34.31–50.73)	70.60 (45.41–283.10)	
Case/control	256/235	230/234	204/235	267/234	
Multivariable-adjusted OR (95% CI)[e]	1.00	0.95 (0.72–1.25)	0.80 (0.61–1.07)	1.05 (0.79–1.39)	0.75
Animal sources					
Mean (range)	2.13 (0.10–2.84)	3.25 (2.80–3.68)	4.10 (3.45–4.64)	5.70 (4.58–11.44)	
Case/control	287/235	223/234	219/235	228/234	
Multivariable-adjusted OR (95% CI)[f]	1.00	0.76 (0.58–1.00)	0.75 (0.57–0.99)	0.75 (0.57–1.00)	0.06
NDEA					
Mean (range)	0.04 (0.008–0.06)	0.06 (0.05–0.07)	0.08 (0.06–0.09)	0.12 (0.09–0.39)	
Case/control	159/235	198/234	268/235	332/234	
Age and energy-adjusted OR (95% CI)	1.00	1.26 (0.96–1.67)	1.72 (1.31–2.25)	2.16 (1.66–2.82)	<0.0001
Multivariable-adjusted OR (95% CI)[d]	1.00	1.35 (1–1.82)	1.89 (1.41–2.53)	2.28 (1.71–3.04)	<0.0001
Plant sources					
Mean (range)	0.01 (<0.001–0.02)	0.02 (0.01–0.03)	0.03 (0.02–0.04)	0.05 (0.03–0.15)	
Case/control	168/235	210/234	280/235	299/234	
Multivariable-adjusted OR (95% CI)[e]	1.00	1.27 (0.94–1.70)	1.66 (1.24–2.22)	1.93 (1.44–2.60)	<0.0001
Animal sources					
Mean (range)	0.02 (<0.001–0.03)	0.04 (0.03–0.05)	0.05 (0.04–0.06)	0.09 (0.06–0.38)	
Case/control	224/235	189/234	219/235	325/234	
Multivariable-adjusted OR (95% CI)[f]	1.00	0.82 (0.61–1.09)	0.97 (0.73–1.29)	1.35 (1.03–1.78)	0.004
NDMA					
Mean (range)	0.28 (0.09–0.41)	0.45 (0.36–0.70)	0.58 (0.47–0.96)	0.99 (0.62–3.45)	
Case/control	239/235	239/234	230/235	249/234	
Age and energy-adjusted OR (95% CI)	1.00	1.00 (0.77–1.29)	0.99 (0.77–1.28)	1.09 (0.84–1.41)	0.47
Multivariable-adjusted OR (95% CI)[d]	1.00	0.99 (0.75–1.31)	1.03 (0.78–1.37)	1.03 (0.78–1.37)	0.78
Plant sources					
Mean (range)	0.04 (<0.001–0.05)	0.06 (0.04–0.07)	0.08 (0.05–0.10)	0.12 (0.07–0.37)	
Case/control	158/235	227/234	300/235	272/234	
Multivariable-adjusted OR (95% CI)[e]	1.00	1.48 (1.10–1.99)	1.97 (1.48–2.64)	1.93 (1.42–2.61)	<0.0001
Animal sources					
Mean (range)	0.18 (0.02–0.33)	0.33 (0.25–0.56)	0.43 (0.35–0.74)	0.74 (0.46–3.37)	
Case/control	250/235	234/234	201/235	272/234	
Multivariable-adjusted OR (95% CI)[f]	1.00	0.97 (0.74–1.28)	0.87 (0.65–1.15)	1.17 (0.89–1.54)	0.26
NDBA[g]					
Mean (range)	0.80 (<0.001–1.35)	1.73 (1.22–2.18)	2.69 (1.96–3.43)	4.54 (2.96–13.76)	
Case/control	279/235	249/234	233/235	196/234	
Age and energy-adjusted OR (95% CI)	1.00	0.90 (0.71–1.16)	0.84 (0.65–1.08)	0.71 (0.55–0.92)	0.01
Multivariable-adjusted OR (95% CI)[d,f]	1.00	0.87 (0.66–1.14)	0.85 (0.65–1.21)	0.64 (0.48–0.85)	0.003
NPYR					
Mean (range)	0.09 (0.02–0.11)	0.13 (0.11–0.14)	0.16 (0.14–0.20)	0.25 (0.19–0.79)	
Case/control	250/235	226/234	262/235	219/234	
Age and energy-adjusted OR (95% CI)	1.00	0.91 (0.71–1.18)	1.05 (0.82–1.35)	0.88 (0.68–1.14)	0.48
Multivariable-adjusted OR (95% CI)[d]	1.00	0.85 (0.65–1.13)	0.97 (0.74–1.27)	0.79 (0.60–1.05)	0.18
Plant sources					
Mean (range)	0.03 (0.006–0.04)	0.04 (0.036–0.05)	0.05 (0.04–0.06)	0.07 (0.06–0.16)	
Case/control	240/235	211/234	255/235	251/234	
Multivariable-adjusted OR (95% CI)[e]	1.00	1.06 (0.80–1.41)	1.22 (0.92–1.60)	1.07 (0.81–1.42)	0.57
Animal sources					
Mean (range)	0.04 (0.002–0.06)	0.07 (0.06–0.09)	0.10 (0.08–0.12)	0.16 (0.11–0.76)	
Case/control	261/235	255/234	213/235	228/234	
Multivariable-adjusted OR (95% CI)[f]	1.00	0.93 (0.71–1.22)	0.76 (0.58–1.00)	0.77 (0.58–1.02)	0.04
Nitrate (NO_3)[h]					
Mean (range)	21.67 (3.67–29.39)	34.37 (27.75–40.42)	45.54 (37.11–53.44)	73.29 (47.62–286.87)	
Case/control	258/235	236/234	192/235	271/234	

Table 2. Continued

N-nitroso compounds from dietary sources	Quartiles of intake[a]				P_{trend}[b]
	Q1	Q2	Q3	Q4	
Total N-nitroso compounds[c]					
Age and energy-adjusted OR (95% CI)	1.00	0.93 (0.72–1.20)	0.76 (0.59–0.99)	1.08 (0.84–1.39)	0.55
Multivariable-adjusted OR (95% CI)[d]	1.00	0.93 (0.71–1.23)	0.72 (0.55–0.96)	1.07 (0.81–1.41)	0.67
Plant sources					
Mean (range)	18.97 (0.56–26.59)	31.43 (24.68–37.25)	42.58 (34.31–50.72)	70.59 (45.40–283.09)	
Case/control	256/235	230/234	204/235	267/234	
Multivariable-adjusted OR (95% CI)[e]	1.00	0.95 (0.72–1.25)	0.80 (0.61–1.07)	1.05 (0.79–1.39)	0.75
Animal sources					
Mean (range)	1.63 (0.09–2.19)	2.46 (2.08–2.77)	3.05 (2.52–3.44)	4.24 (3.25–8.07)	
Case/control	296/235	196/234	220/235	245/234	
Multivariable-adjusted OR (95% CI)[f]	1.00	0.68 (0.52–0.90)	0.74 (0.56–0.97)	0.82 (0.62–1.08)	0.24
Nitrite (NO_2)[g,h]					
Mean (range)	0.37 (0.01–0.59)	0.70 (0.55–0.84)	0.99 (0.83–1.17)	1.55 (1.16–3.86)	
Case/control	291/235	226/234	225/235	215/234	
Age and energy-adjusted OR (95% CI)	1.00	0.80 (0.62–1.03)	0.77 (0.60–1.00)	0.75 (0.59–0.97)	0.04
Multivariable-adjusted OR (95% CI)[d,f]	1.00	0.83 (0.63–1.09)	0.79 (0.60–1.04)	0.68 (0.51–0.91)	0.01

The mean and range units for all NOCs are µg/1000 kcal/day, except total NOCs, nitrate and nitrite, which used unit of mg/1000kcal/day.

[a]Quartile cutoff points were determined based on the FFQ-specific control's distribution.

[b]P_{trend} was calculated by using the median value of each quartile of NOC consumption as a continuous variable in the multivariable-adjusted model.

[c]Intake of total NOCs capture all 21 individual NOCs, nitrate and nitrite.

[d]The model was adjusted for total calorie as continuous values, and age group, sex, race, education level, BMI status, alcohol level, and history of diabetes, smoking status, and family history of pancreatic cancer as categorized in Table I.

[e]Multivariable-adjusted model for NOCs from plant sources additionally adjusted for red and processed meet intake (classified as four-level categorical).

[f]Multivariable-adjusted model for NOCs from animal sources additionally adjusted for total vegetable and fruit intake level (classified as four-level categorical).

[g]Total nitrite consumption was all from animal sources.

very important role in the detoxification of the dietary mutagen heterocyclic aromatic amines (38). It is attempting to speculate that consumption of fermented foods may contribute to the NOC association with cancer not only as a dietary source of exposure but also as a modifier of endogenous NOC formation and metabolism. Because of the low concentration of NOCs in majority of commonly consumed foods and limited food items assayed for NOCs, our study was not able to test the effect of NOC from each different food item or tease out the potential effects of individual food components that contributed to the association of NDEA/NDMA with pancreatic cancer. It is reasonable to believe that this positive association was not explained by known potential confounders, such as total energy or meat intake. Our findings for a positive association of NDEA and NDMA with pancreatic cancer risk need to be replicated in other studies, particularly in prospective cohort studies where dietary data ascertained years prior to symptoms or diagnosis are both readily available and less susceptible to recall bias and/or reverse causality. If confirmed, this finding would provide supporting evidence for the carcinogenic role of specific dietary NOCs in human pancreatic cancer.

Our null findings for nitrate and total NOC are generally consistent with previous reports of four prospective cohort studies that have been published thus far on NOC and pancreatic cancer (14–17). Null associations with overall nitrate or nitrite intake were consistently found in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort of male smokers in Finland with 167 pancreatic cancer cases over 9 years follow-up (16); the Netherlands cohort with 350 pancreatic cancer cases over 13 years of follow-up (17); the Iowa Women's Health Study cohort with 313 cases over 21 years of follow-up (15) and the NIH-American Association of Retired Persons Diet and Health (NIH-AARP) cohort with 1728 cases after 10 years of follow-up

(14). Notably, a positive association and significant trend was observed for the highest intake of nitrite from processed meat in the Iowa Women's Health Study ($HR_{p95\ percentile\ versus\ Q1}$ = 1.66, 95% CI = 1.00–2.75, P_{trend} = 0.05) (15) and the NIH-AARP cohort ($HR_{Q5\ versus\ Q1}$ = 1.18, 95% CI = 0.95–1.47; P_{trend} = 0.11) (14).

Cured or processed meat, particularly from red meat, is a major food source of NOC. Previously, a meta-analysis of 18 prospective cohort studies, assessing diet several years before diagnosis, demonstrated an overall null association between processed meat (RR = 1.09, 95% CI = 0.96–1.23) and red meat (RR = 1.12, 95% CI = 0.98–1.28) with pancreatic cancer risk (39). Consistent with this, no association for processed and/or red meat intake and pancreatic cancer was recently reported in the US Cancer Prevention Study II cohort with 1156 cases over 10 years of follow-up (40). Taken overall, these prospective findings suggest that meat intake *per se* may not be strongly associated with pancreatic cancer, but that mutagens from processed meat and other dietary sources may warrant further investigation with improved exposure assessment.

Our study found an inverse association of nitrite and other processed meat-derived compounds (NDBA and NYPR) with pancreatic cancer, which could largely be explained by the reverse causation phenomena. Pancreatic cancer cases are often diagnosed at late stage with severe though non-specific symptoms such as impaired glucose level, loss of appetite, jaundice, fatigue, nausea and abdominal pain, which could make the patient largely avoid fat intake and selected foods (41,42). For example, a lower intake of red and processed meat near diagnosis would lead to an underestimated level of prior or longer-term exposure to nitrite and some individual NOCs (e.g. NDBA and NPYR) (5).

The association of NOC and pancreatic cancer could be modified by the nitrosation inhibitors (e.g. vitamin C and E) present in the plant foods (14). In a recently published cohort

Table 3. Multivariable-adjusted ORs and 95% CIs of pancreatic cancer risk by quartiles of energy-adjusted food group intake (g/1000 kcal/day)

	Quartiles of intake[a]				
Food groups	Q1	Q2	Q3	Q4	P_{trend}[b]
Processed meat[c]					
Mean (range)	0.77 (0–3.45)	4.08 (2.28–8.57)	6.78 (4.85–11.26)	16.75 (8.52–122.33)	
Case/control	269/235	250/234	155/235	283/234	
Multivariable-adjusted OR (95% CI)[d]	1.00	0.87 (0.66–1.15)	0.56 (0.42–0.76)	0.93 (0.70–1.22)	0.73
Processed red meat[e]					
Mean (range)	0.80 (0–3.45)	3.69 (2.28–5.93)	6.69 (4.85–9.66)	16.13 (8.52–122.33)	
Case/control	282/235	207/234	191/235	277/234	
Multivariable-adjusted OR (95% CI)[d]	1.00	0.72 (0.55–0.96)	0.64 (0.48–0.85)	0.87 (0.66–1.15)	0.64
Red meat[f]					
Mean (range)	16.84 (0–36.68)	33.36 (23.78–52.78)	47.63 (37.26–68.41)	77.08 (55.01–211.84)	
Case/control	289/235	229/234	227/235	212/234	
Multivariable-adjusted OR (95% CI)[d]	1.00	0.78 (0.59–1.03)	0.79 (0.60–1.04)	0.65 (0.48–0.86)	0.005
Total red and processed meat[g]					
Mean (range)	16.87 (0–36.99)	33.27 (23.78–53.08)	48.03 (37.26–70.35)	77.53 (55.01–211.84)	
Case/control	285/235	225/234	235/235	212/234	
Multivariable-adjusted OR (95% CI)[d]	1.00	0.78 (0.59–1.03)	0.82 (0.62–1.07)	0.65 (0.49–0.87)	0.008
Vegetables[h]					
Mean (range)	41.65 (0–61.02)	73.91 (56.33–90.78)	105.98 (87.83–132.48)	178.25 (122.37–454.91)	
Case/control	265/235	247/234	226/235	219/234	
Multivariable-adjusted OR (95% CI)[d]	1.00	0.91 (0.69–1.20)	0.81 (0.61–1.07)	0.75 (0.56–1.00)	0.04
Leafy green vegetables[i]					
Mean (range)	11.45 (0–18.51)	24.09 (11.3–30.6)	37.67 (26.19–47.64)	74.45 (37.92–345.30)	
Case/control	275/235	241/234	202/235	239/234	
Multivariable-adjusted OR (95% CI)[d]	1.00	0.84 (0.64–1.11)	0.69 (0.52–0.92)	0.74 (0.55–0.99)	0.04

[a] Quartile cutoff points were determined based on the FFQ-specific control's distribution.

[b] P_{trend} was calculated by using the median value of each quartile of food group consumption as a continuous variable in the multivariable-adjusted model.

[c] Processed meat in this study included bacon, hotdog, sausage, salami, bologna, and chicken and turkey hot dogs.

[d] The model was adjusted for total calorie as continuous value, and age group, sex, race, education level, BMI status, alcohol level, and history of diabetes, smoking status and family history of pancreatic cancer as categorized in Table I. All relevant meat groups were adjusted simultaneously for each other (i.e. white versus red; processed versus non-processed, processed red meat versus non-processed and/or white meat, fruit and vegetable group versus meat group).

[e] Processed red meat in this study included bacon, hotdog, sausage, salami and bologna.

[f] Total red meat included processed red meat as listed in *e* and non-processed red meats that are all types of beef and pork, hamburger and liver.

[g] Total red and processed meat is inclusive of all red meat and processed meat.

[h] Vegetables did not include juices.

[i] Leafy green vegetables included broccoli, cabbage or cole slaw, brussels sprouts, cooked or raw spinach, kale, iceberg lettuce, romaine lettuce.

study (the European Prospective Investigation of Cancer–Norfolk cohort) of 86 cases over 13 years of follow-up, the effect of red meat intake on pancreatic cancer was attenuated by a high plasma levels of vitamin C (43). Although our study found an inverse association for vegetable intake and pancreatic cancer, no association with dietary nitrate or interaction of NOC with vitamin C and vitamin E was found. Notably, we had limited statistical power in the interaction test; thus, future studies with larger sample size are needed to further verify.

Our study has several strengths. First, adequate sample size of cases allowed us to detect main association between NOCs and related food groups and pancreatic cancer. Second, the pancreatic cancer outcome in our study was clearly defined and accurately ascertained. Third, detailed information on pancreatic cancer risk factors allowed for refined adjustment in the analyses. Fourth, use of validated FFQs and assessment of a variety of food sources pertinent to the application of a relatively comprehensive and validated *N*-nitroso database enabled us to estimate and assess a wide range of different NOCs. Our findings for NDEA and NDMA suggest that assessment of multiple individual NOCs may have certain advantages over the proxy nitrite or nitrate measurement in examining associations with pancreatic cancer. Despite these strengths, study limitations also are noted. For example, the study is subject to the inherited limitations of a case–control study, such as differential misclassification of exposure due to recall bias and reverse causality. The number of cases in the stratified analyses was not large, which limited the statistical power to detect interaction. The *N*-nitroso database was developed based on available literature; therefore, the coverage of food items and NOC content in the database may not be complete. As imputation was used to link food from FFQ to the database and estimate NOC concentration for each food item, there will be some measurement errors in estimating NOC intake. However, this imputation would have been uniform across all cases and controls. The measurement error in FFQ data and other self-reported covariates is another unavoidable limitation. FFQ and database-related limitations may lead to non-differential misclassification of exposure among cases and controls, which possibly biased our results toward null. Although we adjusted for potential confounding factors in the model, residual confounding is still possible. Furthermore, our dietary data collected from 2002 to 2009 may not reflect current US dietary habits or NOC levels, a common limitation in large, established prospective cohorts and case–control studies. However, it is also unlikely that our study participants were exposed to any of the recent increased attention surrounding processed meat intake and cancer risk (2,9) or that they would be aware of the different types of foods containing a range of NOCs (beyond processed meats), which may modulate cancer risk.

In summary, in this large hospital-based matched case–control study, we found a biologically plausible positive

Table 4. Energy-adjusted correlation coefficients of NOC intake and the top five most significant food contributors by case status

Compounds	1	2	3	4	5
Total NOC					
Case	Green leafy vegetables ($\rho = 0.89$)	Other vegetables[a] ($\rho = 0.49$)	Roots[b] ($\rho = 0.39$)	Fresh seafood[c] ($\rho = 0.25$)	Beans and pulses ($\rho = 0.16$)
Control	Green leafy vegetables ($\rho = 0.89$)	Other vegetables[a] ($\rho = 0.41$)	Roots[b] ($\rho = 0.29$)	Chicken ($\rho = 0.21$)	Fresh seafood[c] ($\rho = 0.21$)
NDEA					
Case	Fermented cheese[d] ($\rho = 0.60$)	Fresh seafood[c] ($\rho = 0.41$)	Grains[e] ($\rho = 0.38$)	Salmon ($\rho = 0.32$)	Green leafy vegetables ($\rho = 0.24$)
Control	Grains[e] ($\rho = 0.54$)	Fermented cheese[d] ($\rho = 0.48$)	Fresh seafood ($\rho = 0.42$)	Salmon ($\rho = 0.26$)	Green leafy vegetables ($\rho = 0.20$)
NDMA					
Case	Pizza ($\rho = 0.75$)	Beer ($\rho = 0.37$)	Salmon ($\rho = 0.30$)	Fresh seafood ($\rho = 0.21$)	Wine ($\rho = 0.19$)
Control	Pizza ($\rho = 0.67$)	Beer ($\rho = 0.45$)	Salmon ($\rho = 0.29$)	Fresh seafood ($\rho = 0.18$)	Liquor ($\rho = 0.17$)
Nitrate					
Case	Green leafy vegetables ($\rho = 0.89$)	Other vegetables[a] ($\rho = 0.50$)	Roots[b] ($\rho = 0.39$)	Fresh seafood ($\rho = 0.25$)	Beans and pulses ($\rho = 0.16$)
Control	Green leafy vegetables ($\rho = 0.90$)	Other vegetables[a] ($\rho = 0.42$)	Roots ($\rho = 0.28$)	Chicken ($\rho = 0.21$)	Fresh seafood ($\rho = 0.21$)
Nitrite					
Case	Beef ($\rho = 0.90$)	Cured lunch ($\rho = 0.47$)	Hotdog ($\rho = 0.36$)	Bacon ($\rho = 0.31$)	Eggs ($\rho = 0.16$)
Control	Beef ($\rho = 0.91$)	Cured lunch ($\rho = 0.47$)	Hotdog ($\rho = 0.35$)	Bacon ($\rho = 0.33$)	Eggs ($\rho = 0.18$)
NDBA					
Case	Beef ($\rho = 0.99$)	Cured lunch ($\rho = 0.24$)	Bacon ($\rho = 0.17$)	Hotdog ($\rho = 0.14$)	Roots[b] ($\rho = 0.11$)
Control	Beef ($\rho = 0.99$)	Cured lunch ($\rho = 0.27$)	Bacon ($\rho = 0.21$)	Hotdog ($\rho = 0.17$)	Roots[b] ($\rho = 0.14$)
NPYR					
Case	Chicken ($\rho = 0.48$)	Bacon ($\rho = 0.48$)	Wine ($\rho = 0.38$)	Eggs ($\rho = 0.29$)	Cured lunch ($\rho = 0.22$)
Control	Chicken ($\rho = 0.56$)	Wine ($\rho = 0.41$)	Bacon ($\rho = 0.36$)	Eggs ($\rho = 0.25$)	Green leafy vegetables ($\rho = 0.21$)

Spearman correlation test was conducted using data from 1545 subjects who completed the old version of Harvard FFQ and all correlation coefficients in this table have P values <0.0001.

[a]Other vegetables referred to those non-green leafy vegetables such as zucchini and peas.

[b]Roots referred to yams in the study.

[c]Fresh seafood included shrimp, other fishes and tuna in this study.

[d]Fermented cheese in this study included cottage cheese, cream cheese, American cheese, sour cream and yogurt.

[e]Grains in this study included brown rice, white rice, chocolate chip, farina (creamed wheat), cookies and bars, oatmeal, bran flakes (breakfast cereal), rye bread, dark bread, muffins, pancake, pasta, popcorn, rolls or sweet rolls.

association of two potent dietary carcinogens, NDEA and NDMA, with pancreatic cancer. In addition to a careful examination of potential mechanisms, these findings need to be confirmed in readily available large, prospective cohort studies with consideration of sufficient time between diet assessment to disease diagnosis or symptoms and diagnosis. If confirmed, it will add direct evidence to the carcinogenic role of NOCs in human pancreatic cancer.

Supplementary material

Supplementary data are available at *Carcinogenesis* online.

Funding

References

1. Siegel, R.L. *et al.* (2018) Cancer statistics, 2018. CA. Cancer J. Clin., 68, 7–30.
2. World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer. Continuous Project Export Report 2018. Available at dietandcancerreport.org. (11 August 2018, date last accessed).
3. Pour, P.M. *et al.* (1981) Current knowledge of pancreatic carcinogenesis in the hamster and its relevance to the human disease. Cancer, 47(suppl. 6), 1573–1589.
4. Lijinsky, W. (1999) N-nitroso compounds in the diet. Mutat. Res., 443, 129–138.
5. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (2010) IARC monographs on the evaluation of carcinogenic risks to humans. Ingested nitrate and nitrite, and cyanobacterial peptide toxins. IARC Monogr. Eval. Carcinog. Risks Hum., 94, v–vii, 1–412.
6. Hecht, S.S. (1997) Approaches to cancer prevention based on an understanding of N-nitrosamine carcinogenesis. Proc. Soc. Exp. Biol. Med., 216, 181–191.

7. Tricker, A.R. (1997) *N*-nitroso compounds and man: sources of exposure, endogenous formation and occurrence in body fluids. Eur. J. Cancer Prev., 6, 226–268.

8. Scanlan, R.A. (1983) Formation and occurrence of nitrosamines in food. Cancer Res., 43(suppl. 5), 2435s–2440s.

9. Bouvard, V. *et al*.; International Agency for Research on Cancer Monograph Working Group. (2015) Carcinogenicity of consumption of red and processed meat. Lancet. Oncol., 16, 1599–1600.

10. Risch, H.A. (2003) Etiology of pancreatic cancer, with a hypothesis concerning the role of *N*-nitroso compounds and excess gastric acidity. J. Natl. Cancer Inst., 95, 948–960.

11. Baghurst, P.A. *et al*. (1991) A case–control study of diet and cancer of the pancreas. Am. J. Epidemiol., 134, 167–179.

12. Coss, A. *et al*. (2004) Pancreatic cancer and drinking water and dietary sources of nitrate and nitrite. Am. J. Epidemiol., 159, 693–701.

13. Howe, G.R. *et al*. (1990) Dietary factors and risk of pancreatic cancer: results of a Canadian population-based case–control study. Int. J. Cancer, 45, 604–608.

14. Aschebrook-Kilfoy, B. *et al*. (2011) Pancreatic cancer and exposure to dietary nitrate and nitrite in the NIH-AARP Diet and Health Study. Am. J. Epidemiol., 174, 305–315.

15. Quist, A.J.L. *et al*. (2018) Ingested nitrate and nitrite, disinfection by-products, and pancreatic cancer risk in postmenopausal women. Int. J. Cancer, 142, 251–261.

16. Stolzenberg-Solomon, R.Z. *et al*. (2002) Prospective study of diet and pancreatic cancer in male smokers. Am. J. Epidemiol., 155, 783–792.

17. Heinen, M.M. *et al*. (2009) Meat and fat intake and pancreatic cancer risk in the Netherlands Cohort Study. Int. J. Cancer, 125, 1118–1126.

18. Stuff, J.E. *et al*. (2009) Construction of an *N*-nitroso database for assessing dietary intake. J. Food Compost. Anal., 22(suppl. 1), S42–S47.

19. Bartsch, H. *et al*. (1988) Inhibitors of endogenous nitrosation. Mechanisms and implications in human cancer prevention. Mutat. Res., 202, 307–324.

20. Lunn, J.C. *et al*. (2007) The effect of haem in red and processed meat on the endogenous formation of *N*-nitroso compounds in the upper gastrointestinal tract. Carcinogenesis, 28, 685–690.

21. Hassan, M.M. *et al*. (2007) Risk factors for pancreatic cancer: case–control study. Am. J. Gastroenterol., 102, 2696–2707.

22. Li, D. *et al*. (2006) Polymorphisms of cytochrome P4501A2 and *N*-acetyltransferase genes, smoking, and risk of pancreatic cancer. Carcinogenesis, 27, 103–111.

23. Sakia, R. (1992) The Box-Cox transformation technique: a review. The Statistician, 41, 169–178.

24. Li, D. *et al*. (2009) Body mass index and risk, age of onset, and survival in patients with pancreatic cancer. JAMA, 301, 2553–2562.

25. National Institute on Alcohol Abuse and Alcoholism. *What Is a Standard Drink?* https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/what-standard-drink (4 December 2018, date last accessed).

26. Rimm, E.B. *et al*. (1992) Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am. J. Epidemiol., 135, 1114–26; discussion 1127.

27. Hunter, D.J. *et al*. (1988) Variability in portion sizes of commonly consumed foods among a population of women in the United States. Am. J. Epidemiol., 127, 1240–1249.

28. Willett, W.C. *et al*. (1985) Reproducibility and validity of a semiquantitative food frequency questionnaire. Am. J. Epidemiol., 122, 51–65.

29. Block, G. *et al*. (1994) Revision of dietary analysis software for the Health Habits and History Questionnaire. Am. J. Epidemiol., 139, 1190–1196.

30. Stuff, J.E. *et al*. (2009) *N*-nitroso compounds: assessing agreement between food frequency questionnaires and 7-day food records. J. Am. Diet. Assoc., 109, 1179–1183.

31. Harvard, T.H. Chan School of Public Health Nutrition Department's File Download Site https://regepi.bwh.harvard.edu/health/FFQ/files (5 January 2018, date last accessed).

32. Pearce, N. (2016) Analysis of matched case–control studies. BMJ, 352, i969.

33. Stolzenberg-Solomon, R.Z. *et al*. (2007) Meat and meat-mutagen intake and pancreatic cancer risk in the NIH-AARP cohort. Cancer Epidemiol. Biomarkers Prev., 16, 2664–2675.

34. Zheng, J. *et al*. (2017) Dietary patterns and risk of pancreatic cancer: a systematic review. Nutr. Rev., 75, 883–908.

35. Etemadi, A. *et al*. (2017) Mortality from different causes associated with meat, heme iron, nitrates, and nitrites in the NIH-AARP Diet and Health Study: population based cohort study. BMJ, 357, j1957.

36. Neyman, J. *et al*. (1992) On the problem of the most efficient tests of statistical hypotheses. In Breakthroughs in Statistics. Springer, New York, pp. 73–108.

37. Li, D. (2012) Diabetes and pancreatic cancer. Mol. Carcinog., 51, 64–74.

38. Knasmüller, S. *et al*. (2001) Impact of bacteria in dairy products and of the intestinal microflora on the genotoxic and carcinogenic effects of heterocyclic aromatic amines. Mutat. Res., 480-481, 129–138.

39. Zhao, Z. *et al*. (2017) Association between consumption of red and processed meat and pancreatic cancer risk: a systematic review and meta-analysis. Clin Gastroenterol Hepatol, 15, 486–493.e10.

40. McCullough, M.L. *et al*. (2018) Meat consumption and pancreatic cancer risk among men and women in the Cancer Prevention Study-II Nutrition Cohort. Cancer Causes Control, 29, 125–133.

41. Jansen, R.J. *et al*. (2013) Meat-related mutagens and pancreatic cancer: null results from a clinic-based case-control study. Cancer Epidemiol. Biomarkers Prev., 22, 1336–1339.

42. La Vecchia, C. *et al*. (1990) Medical history, diet and pancreatic cancer. Oncology, 47, 463–466.

43. Beaney, A.J. *et al*. (2017) Higher meat intake is positively associated with higher risk of developing pancreatic cancer in an age-dependent manner and are modified by plasma antioxidants: a prospective cohort study (EPIC-Norfolk) using data from food diaries. Pancreas, 46, 672–678.